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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of:

David MACK, *et al.*

Appl. No. 09/828,307

Filed: April 6, 2001

For: **NOVEL METHODS OF DIAGNOSING CANCER, COMPOSITIONS, AND METHODS OF SCREENING FOR CANCER MODULATORS**

Art Unit: 1637

Examiner: Kim, Young, J.

Atty. Docket: 05882.0142.CPUS03

Confirmation No. 7761

RESPONSE TO OFFICE ACTION

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22213-1450

Sir:

This is in response to the Office Action dated **January 14, 2003**, and is submitted on or before the extended due date of **July 14, 2003**. A petition for a three-month extension of time and its requisite fee are enclosed herewith. The Examiner is requested to enter the amendments and consider the application.

THE AMENDMENTS

In the Specification

Amend the paragraph starting at page 11, line 4:

In a preferred embodiment, differentially expressed sequences are those that are up-regulated in breast cancer and/or colorectal cancer; that is, the expression of these genes is higher in carcinoma as compared to normal breast or colon tissue. "Up-regulation" as used herein means at least about a 50% increase, preferably a two-fold change, more preferably at least about a three fold change, with at least about five-fold or higher being preferred. All accession numbers herein are for the GenBank sequence database and the sequences of the accession numbers are hereby expressly incorporated by reference. GenBank is known in the art, see, e.g., Benson, DA, et al., Nucleic Acids Research 26:1-7 (1998) ~~and http://www.ncbi.nlm.nih.gov/~~. In addition, these genes were found to be expressed in a limited amount or not at all in heart, brain, lung, liver, kidney, muscle, pancreas, testes, stomach, small intestine and spleen.

C1

Amend the paragraph starting at page 15, line 10:

Another example of a useful algorithm is the BLAST algorithm, described in Altschul et al., J. Mol. Biol. 215, 403-410, (1990) and Karlin et al., PNAS USA 90:5873-5787 (1993). A particularly useful BLAST program is the WU-BLAST-2 program which was obtained from Altschul et al., Methods in Enzymology, 266: 460-480 (1996) ~~[http://blast.wustl/edu/blast/ READ.html]~~. WU-BLAST-2 uses several search parameters, most of which are set to the default values. The adjustable parameters are set with the following values: overlap span =1, overlap fraction = 0.125, word threshold (T) = 11. The HSP S and HSP S2 parameters are dynamic values and are established by the program itself depending upon the composition of the particular sequence and composition of the particular database against which the sequence of interest is being searched; however, the values may be adjusted to increase sensitivity. A % amino acid sequence identity value is determined by the number of matching identical residues divided by the total number of residues of the "longer" sequence in the aligned region. The "longer" sequence is the one having the most actual residues in the aligned region (gaps introduced by WU-Blast-2 to maximize the alignment score are ignored).

C2

Amend the paragraph starting at page 58, line16:

Purify total RNA from tissue using ~~TRIzol~~ TRIZOL® Reagent

Estimate tissue weight. Homogenize tissue samples in 1ml of ~~TRIzol~~ TRIZOL® (chemical reagents for use in isolating biological material from organic tissue) per 50mg of tissue using a Polytron 3100 homogenizer. The generator/probe used depends upon the tissue size. A generator that is too large for the amount of tissue to be homogenized will cause a loss of sample and lower RNA yield. Use the 20mm generator for tissue weighing more than 0.6g. If the working volume is greater than 2ml, then homogenize tissue in a 15ml polypropylene tube (Falcon 2059). Fill tube no greater than 10ml.

C3

Amend the paragraph starting at page 59, line 7

Add 0.2ml of chloroform per 1ml of ~~TRIzol~~ TRIZOL® reagent used in the original homogenization. Cap tubes securely and shake tubes vigorously by hand (do not vortex) for 15 seconds. Incubate samples at room temp. for 2-3 minutes. Centrifuge samples at 6500rpm in a Sorvall superspeed for 30 min. at 4°C. (You may spin at up to 12,000 x g for 10 min. but you risk breaking your tubes in the centrifuge.)

C4

Amend the paragraph starting at page 59, line 13:

Transfer the aqueous phase to a fresh tube. Save the organic phase if isolation of DNA or protein is desired. Add 0.5ml of isopropyl alcohol per 1ml of ~~TRIzol~~ TRIZOL® reagent used in the original homogenization. Cap tubes securely and invert to mix. Incubate samples at room temp. for 10 minutes. Centrifuge samples at 6500rpm in Sorvall for 20min. at 4°C.

C5

Amend the paragraph starting at page 59, line 18:

Pour off the supernate. Wash pellet with cold 75% ethanol. Use 1ml of 75% ethanol per 1ml of ~~TRIzol~~ TRIZOL® reagent used in the initial homogenization. Cap tubes securely and invert several times to loosen pellet. (Do not vortex). Centrifuge at <8000rpm (<7500 x g) for 5 minutes at 4°C.

C6

Amend the paragraph starting at page 59, line 27:

Purify poly A+ mRNA from total RNA or clean up total RNA with Qiagen' s ~~RNeasy~~ RNEASY® (chromatographic materials for separation of nucleic acids) kit

Purification of poly A^+ mRNA from total RNA. Heat ~~oligotex~~ OLIGOTEX® (chemicals for the purification of nucleic acids) suspension to 37°C and mix immediately before adding to RNA. Incubate Elution Buffer at 70°C. Warm up 2 x Binding Buffer at 65°C if there is precipitate in the buffer. Mix total RNA with DEPC-treated water, 2 x Binding Buffer, and ~~Oligotex~~ OLIGOTEX® according to Table 2 on page 16 of the ~~Oligotex~~ OLIGOTEX® Handbook. Incubate for 3 minutes at 65°C. Incubate for 10 minutes at room temperature.

C7

Centrifuge for 2 minutes at 14,000 to 18,000 g. If centrifuge has a "soft setting," then use it. Remove supernatant without disturbing ~~Oligotex~~ OLIGOTEX® pellet. A little bit of solution can be left behind to reduce the loss of ~~Oligotex~~ OLIGOTEX®. Save sup until certain that satisfactory binding and elution of poly A^+ mRNA has occurred.

Amend the paragraph at page 60, line 25:

C8

Clean up total RNA using Qiagen's ~~RNeasy~~ RNEASY® (chromatographic materials for separation of nucleic acids) kit

Amend the paragraph starting at page 62, line 23:

~~RNeasy~~ RNEASY® clean-up of IVT product

C9

Follow previous instructions for ~~RNeasy~~ RNEASY® (chromatographic materials for separation of nucleic acids) columns or refer to Qiagen's ~~RNeasy~~ RNEASY® protocol handbook.